Election and Reply to Restriction

to Office Action dated September 15, 2008

Atty. Docket No: 56029-51044

Listing of claims:

1. (Currently Amended) A live attenuated derivative of a pathogenic Salmonella species consisting essentially of

(a) a means for regulatable expression of a <u>fur</u> gene that encodes a regulatory protein, wherein a regulatable promotor is operably linked to said gene, wherein said gene is expressed when said attentuated strain is in the intestinal tract of an individual and said gene is not expressed when said attenuated strain is within internal tissues of an individual and wherein non-expression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved among *Salmonella* species and *E. coli* strains; and

(b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among *Salmonella* species and *E. coli* strains;

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against *Salmonella* species and *E. coli* strains.

- 2. (Previously presented) The live attenuated derivative of claim 1, further comprising a means for non-expression of a serotype-specific antigen.
- 3. (Currently amended) The live attenuated derivative of claim 2, wherein said means for non-expression of a serotype-specific antigen comprises a mutation in a gene selected from the group consisting of *fliC* and *fliB* fliB.
- 4. (Previously presented) The live attenuated derivative of claim 3, wherein said mutation is a deletion mutation.
- 5. (Previously presented) The live attenuated derivative of claim 1, wherein said means of regulatable expression comprises substituting the promoter of said gene that encodes a regulatory protein with a regulatable promoter.

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6. (Previously presented) The live attenuated derivative of claim 5 wherein said regulatable

promoter is the *araCP*_{BAD} repressor-activator-promoter system.

7. (Canceled)

8. (Previously presented) The live attenuated derivative of claim 1 wherein said carbohydrate

antigen is an LPS O-antigen.

9. (Previously presented) The live attenuated derivative of claim 8 wherein said means for

regulatable synthesis comprises a mutation in a gene that encodes a product necessary for

synthesis of LPS O-antigen.

10. (Previously presented) The live attenuated derivative of claim 9, wherein said means for

regulatable synthesis comprises a mutation in the *pmi* gene.

11. (Previously presented) A method for inducing an immune response sufficient for protection

against infection by Salmonella species and E. coli strains, said method comprising

administering to an individual the live attenuated derivative of claim 1.

12. (Currently amended) A live attenuated derivative of a pathogenic Salmonella species,

consisting essentially of

(a) a means for regulatable expression of a fur gene, wherein the fur promoter is replaced

with a regulatable promotor operably linked to said fur gene, wherein said fur gene is expressed

when said attentuated strain is in the intestinal tract of an individual and said fur gene is not

expressed when said attenuated strain is within internal tissues of an individual; and

(b) a mutation that renders a pmi gene inoperable,

wherein said attenuated derivative has enhanced ability to induce cross-protective

immunity against Salmonella species and E. coli.

13. (Canceled)

14. (Previously presented) The live attenuated derivative of claim 12, wherein said means of (a)

comprises replacing the *fur* promoter with the *araCP*_{BAD} activator-repressor-promoter system.

15. (Previously presented) The live attenuated derivative of claim 12 wherein said means of (a)

comprises the ΔP fur:: $araCP_{BAD}$ fur genetic construction.

16. (Previously presented) The live attenuated derivative of claim 12 wherein said mutation of

(b) is a deletion mutation.

17. (Previously presented) A method of inducing a cross-protective immune response against

Salmonella species, said method comprising administering to an individual the live attenuated

derivative of claim 2.

18. (Canceled)

19. (Currently amended) A vaccine comprising a live attenuated strain of Salmonella, wherein

said live attenuated strain consists essentially of

(a) a mutation in a pmi gene that renders said pmi gene non functional; and;

(b) a genetic construction that allows for regulatable expression of a fur gene, wherein

said fur gene is expressed when said attenuated strain is in the intestinal tract of an individual

and said fur gene is not expressed when said attenuated strain is within internal tissues of an

individual, and

wherein said vaccine has enhanced ability to stimulate cross protective immunity against

Salmonella species and E. coli strains.

20. (Canceled)

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21. (Previously presented) A vaccine comprising a live attenuated strain of Salmonella, wherein

said live attenuated strain consists essentially of

(a) a mutation that renders a pmi gene non functional; and

(b) a regulatable promotor operably linked to a *fur* gene wherein said *fur* gene is

expressed when said attenuated strain is in the intestinal tract of an individual and said fur gene

is not expressed when said attenuated strain is within internal tissues of an individual.

22. (Previously presented) The vaccine of claim 21 wherein said regulatable promoter

comprises the araCP_{BAD} activator-repressor-promoter system.

23. (Previously presented) A live attenuated derivative of a Salmonella species consisting

essentially of

(a) a means for regulatable synthesis of LPS O-antigen side chains, wherein said O-

antigen side chains are synthesized when said attenuated derivative is in the intestinal tract of an

individual and are not synthesized when said attenuated derivative is within internal tissues of an

individual; and

(b) a means for regulatable expression of a fur gene, wherein said fur gene is expressed

when said attenuated derivative is in the intestinal tract of an individual and wherein said fur

gene is not expressed when said attenuated derivative within internal tissues of an individual

wherein said attenuated derivative has increased ability to induce cross-protective

immunity against infection by Salmonella species and E. coli strains.

24. (Previously presented) The live attenuated derivative of claim 23 wherein said means for

regulatable synthesis comprises a mutation in a gene that encodes a product necessary for

synthesis of LPS O-antigens.

25. (Previously presented) The live attenuated derivative of claim 24 wherein said gene that

encodes a product necessary for synthesis of LPS O-antigens is a pmi gene.

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26. (Currently amended) [[A]] The live attenuated derivative of claim 1, wherein said

pathogenic Salmonella species is a Salmonella typhimurium comprising

(a) a ΔPfur::TTaraCP_{BAD}fur deletion-insertion mutation; and

(b) a Δpmi mutation.

27. (Currently amended) The live attenuated derivative of claim 1, A recombinant bacterial

strain consisting essentially of a means of regulatable expression of wherein said gene of (a) is a

virulence gene[[,]] and wherein said regulatable expression of [[a]] said virulence gene renders

said bacterial strain attenuated while maintaining immunogenicity.

28. (Currently amended) The <u>live attenuated derivative</u> recombinant Salmonella of claim 27,

wherein said virulence gene is selected from the group consisting of aroA, aroC, aroD, cya, crp,

cdt, ompR, htrA, hemA, purA, purB, rfa, rfb, asd ompC and ompF.

29. (Currently amended) The live attenuated derivative recombinant bacterial strain of claim

27, wherein said means of regulatable expression comprises substituting the promoter for said

virulence gene with the araCP_{BAD} repressor-activator-promoter system.

30. (Currently amended) The live attenuated derivative recombinant bacterial strain of claim

29, wherein said virulence gene is a fur gene.

31. (Currently amended) The live attenuated derivative recombinant bacterial strain of claim

30, further comprising a Δpmi mutation.

32. (Currently amended) [[A]] The live attenuated derivative of claim 1 a pathogenic

Enterobacteriaceae species consisting essentially of a ΔP fur:: $araCP_{BAD}$ fur genetic construction.

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33. (Currently amended) A live attenuated derivative of a pathogenic Salmonella species

consisting essentially of

(a) a means for regulatable expression of a gene that encodes a regulatory protein,

wherein a regulatable promotor is operably linked to said gene, wherein said gene is expressed

when said attentuated strain is in the intestinal tract of an individual and said gene is not

expressed when said attenuated strain is within internal tissues of an individual and wherein non-

expression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved

among Salmonella species and E. coli strains; and

(b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first

carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen

that is conserved among Salmonella species and E. coli strains; and

(c) a mutation of fliC or fljB, wherein said mutation results in deletion of the variable

domain while retaining the N-terminal and C-terminal constant domains of flagellar proteins;

wherein said attenuated derivative has enhanced ability to induce cross-protective

immunity against Salmonella species and E. coli strains.

34. (Previously presented) The live attenuated derivative of claim 1, further comprising a means

for biological containment.

35. (Previously presented) The live attenuated derivative of claim 34, wherein said means

comprises a mutation that abolishes motility, prevents synthesis of the exopolysaccharide colanic

acid, prevents synthesis of components of the bacterial extracellular matrix, reduces ability to

withstand the stresses of stationary phase and starvation, reduces ability to use nucleic acids as a

nutrient, or uncouples regulation of cellular activities from a dependence on protein synthesis.

36. (Currently amended) The live attenuated derivative of claim 35, wherein said mutation is

selected from the group consisting of $\Delta(gmd\text{-}fcl)$ -26, $\Delta agfBAC811$, $\Delta bcsABZC2118$,

 $\Delta bcsEFG231$ 9 $\Delta bcsABZC2119$, $\Delta adrA1418$, $\Delta mlrA34$, $\Delta yhiR36::TT$, $\Delta endA2311$, $\Delta relA1123$.

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37. (Previously presented) The live attenuated derivative of claim 35, wherein said mutation consists of a mutation in a gene selected from the group consisting of gmd, fcl, agf, bcs, adr, mlr, yhi, end and rel.

38. (Previously presented) The live attenuated derivative of claim 1, further comprising a mutation in a gene selected from the group consisting of sip and sop.

39. (Previously presented) The live attenuated derivative of claim 38, wherein said mutation is $\triangle sop B1925$.

40. (Previously presented) The live attenuated derivative of claim 1, wherein said live attenuated derivative comprises the $\Delta ilvG3$::TTaraCP_{BAD}lacI genetic construction.